

Sustained major molecular response on interferon alpha-2b in two patients with polycythemia vera

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Abstract Quantitative assessment of the JAK2 V617F allele burden during disease evolution and ongoing myelosuppressive treatment is likely to be implemented in the future clinical setting. Interferon alpha has demonstrated efficacy in treatment of both chronic myeloid leukemia and the Philadelphia chromosome negative chronic myeloproliferative disorders. Reductions in the JAK2 V617F allele burden in patients treated with pegylated interferon alpha-2a (Peg-IFN-2a) have been demonstrated, although follow-up was relatively short. We report here the first profound and sustained molecular responses with a JAK2 V617F allele burden below 1.0% in two patients with polycythemia vera treated with interferon alpha-2b (IFN-2b). Discontinuation of IFN-2b in one of the patients was followed by a sustained long-lasting (12 months of follow-up) major molecular response.

Keywords Polycythemia vera · Interferon · PCR · JAK2 · Remission

Introduction

Quantitative assessment of the JAK2 V617F allele burden is possible by real-time quantitative polymerase chain reaction (qPCR). Monitoring of the JAK2 V617F clonal evolution during disease progression and ongoing myelosuppressive treatment is likely to be implemented in the future clinical setting. Interferon alpha has demonstrated efficacy in both chronic myeloid leukemia (CML) [1] and the Philadelphia chromosome negative chronic myeloproliferative disorders (Ph⁻CMPD) [2, 3]. Major and complete cytogenetic and molecular responses have been reported in patients with CML [1], and rare observations of eradication of chromosomal abnormalities have been reported in Ph⁻CMPD as well [4]. Published data suggest the efficacy of interferon alpha-2a in reducing the JAK2 V617F allele burden. In the study by Kiladjian et al., a substantial proportion of patients had significant reductions in the JAK2 V617F allele burden, and in one patient of the 27 treated with pegylated interferon alpha-2a (Peg-IFN-2a) undetectable levels of JAK2 V617F were recorded after 12 months on treatment [5]. However, data on interferon alpha-2b have demonstrated only very modest molecular responses, but follow-up has been rather short [6–8]. Although only information on treatment with “IFN-alpha2” is given in the report by Jones et al., we assume that all their patients have been treated with IFN-alpha2b and not IFN-alpha2a or being interchanged [7]. This report describes the first profound and sustained molecular responses with a JAK2 V617F allele burden below 1.0% in two patients with

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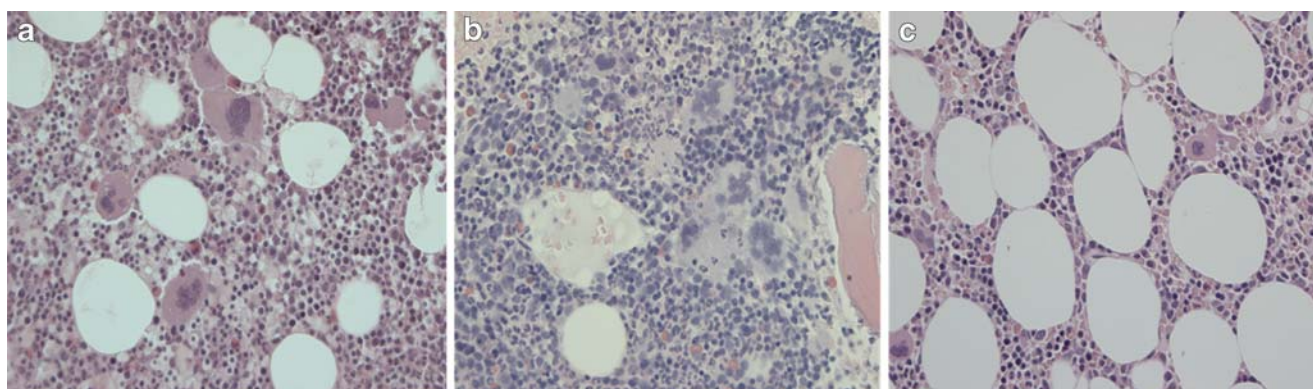


Fig. 1 Bone marrow histomorphology from patient 1 at **a** time of diagnosis 1996 and **b** just prior to treatment with IFN-alpha2b. Both panels demonstrate classical PV features with hypercellularity and clustering of morphological abnormal megakaryocytes. Panel **c** shows

the morphologically normal bone marrow from August 2007 (after 8 years of treatment with IFN-alpha2b) with total regression of PV features

polycythemia vera (PV) on long-term treatment with interferon alpha-2b (IFN-2b).

Methods and results

In our out-patient clinic, five patients have been treated long term (>36 months) with interferon alpha-2b. Two out of these five patients had remarkable responses.

Patient 1 was a 59-year-old male diagnosed with PV in 1996. The patient had a history of a transient ischemic attack. The initial hemoglobin concentration was 19.7 g/dl and the hematocrit was 0.60. The platelet count was slightly elevated and white blood cell counts were normal or marginally increased. The patient had a modest splenomegaly. The bone marrow biopsy showed classical features of PV (Fig. 1). Treatment with IFN-2b ($3\text{--}5 \times 10^6$ IE three times per week) was initiated in 1999. In March 2003, the patient switched

to pegylated IFN-2b (30 μg once weekly, and from January 2005 40 μg once every second week). The treatment was well tolerated and the patient is still kept on the drug without side effects. The patient has been in complete hematological remission without phlebotomy requirements since January 2005. Eight samples of unfractionized peripheral white blood cells collected from July 2004 to August 2007 were all negative for the JAK2 V617F mutation when analyzed by allele-specific PCR [9]. All samples were reanalyzed by the more sensitive (0.1%) qPCR assay designed by our own group and described previously [10]. Briefly, the ratio of JAK2 V617F and wild-type alleles was determined by two allele-specific quantitative real-time PCR assays run in parallel. The qPCR assay sensitivity was calculated from repeated amplifications of wild-type donor DNA from 50 healthy donors by both the wild-type and V617F mutation-specific primers. ($\Delta\text{CT}=14$, corresponding to 1:10,000 or 0.01%). However, we defined a tenfold-higher cut-off limit,

Table 1 The JAK2 V617F allele burden in peripheral blood (PB) and bone marrow (BM) aspirate and biopsy at the different time points during the 11-year follow-up period

Time point	JAK2 V617F %					
	PB leukocytes		BM aspirate		BM biopsy	
	Patient 1	Patient 2	Patient 1	Patient 2	Patient 1	Patient 2
May 1996	(-)	(-)	70	78	54	78
Feb 1999	(-)	(-)	70	(-)	73	(-)
Sep 2004	0.1	(-)	(-)	(-)	(-)	(-)
Jan 2005	0.3	(-)	(-)	(-)	(-)	(-)
July 2005	0.3	(-)	(-)	(-)	(-)	(-)
Nov 2005	0.1	(-)	(-)	(-)	(-)	(-)
Mar 2006	0.4	(-)	(-)	(-)	(-)	(-)
July 2006	0.2	(-)	(-)	(-)	(-)	(-)
Nov 2006	(-)	1	(-)	(-)	(-)	(-)
Mar 2007	0.4	1	(-)	(-)	(-)	(-)
Aug 2007	0.5	1	0.5	(-)	0.5	(-)

(-) No sample available

corresponding to 1:1,000 (0.1%), to be significant of JAK2 V617F allele detection. A small JAK2 V617F allele burden in the range of 0.1–0.5% was detectable in all samples. Next, we analyzed the archived bone marrow samples from diagnosis (1996) and prior to treatment with IFN-2b (1999) as well as in a new bone marrow sample (August 2007). The JAK2 V617F allele burden was in the range of 54% and 73% in both archived samples. In the bone marrow sample from August 2007, the JAK2 V617F allele burden comprised only 0.5%, equivalent to the amount measured in peripheral blood. There were no morphological features of PV (Fig. 1).

Patient 2 was a 35-year-old male diagnosed in 1996. The initial hemoglobin concentration was 19.5 g/dl and the hematocrit was 0.56. The white blood cell count was $10 \times 10^9/l$ and the platelet count was $731 \times 10^9/l$. The spleen was not enlarged. The patient suffered from modest pruritus but was otherwise without complaints. From 1996 to August 2006, the patient was treated with IFN-2b ($3\text{--}5 \times 10^6$ IE three times a week). In August 2006, the treatment was stopped during hospitalization for an acute myocardial infarction. The patient needed phlebotomy three to four times a year on average until December 2003. Since then he has been in a sustained complete hematological remission without phlebotomy requirements. In three consecutive blood samples from November 2006, March and August 2007, a JAK2 V617F allele burden of 1% was demonstrated (Table 1). The bone marrow sample from the time of diagnosis in 1996 showed a JAK2 V617F allele burden of 78%. The patient has now been off from treatment for 12 months and has remained in complete hematological remission with a sustained molecular response with a JAK2 V617F allele burden below 1%.

Discussion

We provide here the first evidence of a major and near complete molecular response in two PV patients with clinical evidence of remission after 8 years of IFN-2b treatment. Although a unified definition of molecular remission in JAK2 V617F positive disorders remains to be established, and the JAK2 V617F clones remained detectable at levels at the border of our qPCR sensitivity limit (0.1%) and up to 1%, they were undetectable with one of the conventionally most widely used assays [9]. Previously, we have demonstrated that the JAK2 V617F allele burden in peripheral blood leukocytes is a reliable and exact measurement of the JAK2 V617F mutated clone in the bone marrow [11]. Unfortunately, we did not have access to either bone marrow or peripheral blood samples throughout the follow-up period. Accordingly, a firm conclusion on the time needed to obtain a major molecular response is impossible. One can speculate that several years of treat-

ment with IFN-2b is necessary for obtaining a sustained profound molecular response and that the previous reports on molecular responses after Peg-IFN-2b and Peg-IFN-2a are weakened by a too short follow-up [5–8]. It is most interesting that the JAK2 V617F allele burden did not expand in patient 2 during the 12-month period, in which the patient did not receive myelosuppressive treatment. This observation is in contrast with what has been published by Ishii et al., who reported a prompt reemergence of the JAK2 V617F clone and clinical symptoms of PV after discontinuation of treatment with IFN-2a for 3 months [12]. Although remarkable, 12 months of treatment without re-appearance of signs of PV or increasing JAK2 V617F allele burden does not exclude a late relapse.

In conclusion, we have for the first time shown that long-term treatment of PV with interferon alpha-2b can induce deep or “near complete” molecular responses, accompanied by a complete morphological remission in the bone marrow in a subset of patients. Furthermore, this report documents that discontinuation of interferon alpha may be followed by a long period of at least a year in which a major molecular response may be sustained. However, it is likely that long-term treatment (several years) is necessary for obtaining such responses. Our observations support the contention of up-front treatment with interferon alpha in patients with JAK2-positive PV, since this treatment strategy may induce a major and sustained molecular response with normalization of the bone marrow in a subgroup of patients. Our findings call for prospective studies with serial measurements of the JAK2 V617F allele burden to assess the efficacy of interferon alpha (2a and 2b) as up-front treatment of PV with the ultimate objective to induce major molecular responses and consequently and hopefully also to reduce the morbidity associated with this disorder—thrombohemorrhagic complications, leukemic and myelofibrotic transformation.

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